Biotechnology Research and Policy Activities of ABSP In Indonesia

1991--2002

ABSP

The Agricultural Biotechnology Support Project

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Contents

Micropropagation of tropical crops for commercial planting (Indonesia)

Fitotek Unggul, Indonesia
DNA Plant Technology (DNAP), USA

1991-1996

Project Goal

The impetus for this project came from Fitotek Unggul, a small Indonesian tissue culture company because they were receiving large orders for pineapple plantlets for Indonesian plantations. Conventional production of pineapple, although less costly than other means, could not keep pace with the demand, and tissue culture offered a cost-effective method to provide disease-free seedlings year around. The primary goal of this project was to develop methods for pineapple micropropagation in liquid cultures, to transfer the technology to Fitotek, and to begin the process of commercialization.

Project Activities

This collaborative project capitalized on initial development of bioreactor technology for axillary shoot bud multiplication of pineapple in a liquid culture system. It was hoped that this method would be Fitotek's answer for further growth in anticipation of a demand for more than 15 million plants per year. DNAP had extensive experience in tissue culture/regeneration in a large number of plant species, including major tropical plantation crops, and Fitotek had been in the business of plant propagation for several years and was able to contribute expertise in commercial micropropagation and sales networking for the target crops. The companies cooperated successfully to maximize the benefits of advanced micropropagation methods and to actively market the products of these ventures.

Project Impacts

- In its first uses of the bioreactor for mass propagation, Fitotek was able to use 32 initial shoots to produce 3,400 harvestable plants with greater consistency of size and color.
- The bioreactor system was effective for commercial production with the potential of producing pineapple plants 40% more cheaply than previous methods. This system effectively reduced requirements for labor, raw materials, electricity and space.
- With these encouraging results, Fitotek added several bioreactor units with the capacity of producing 12 million plantlets a year. However, the demand for pineapple declined rapidly during the project, and necessitated a reassessment of this strategy. At the same time, demand for ginger in the country was rising, and Fitotek was able to adapt its micropropagation techniques to produce ginger instead of pineapple.
- This project illustrates a successful collaboration between private sector partners, and the flexibility of the partners to meet changing market demands.

Development of Asian corn borer resistance in tropical maize

Garst Seed Company (formerly ICI Seeds, Inc.), USA
Central Research Institute for Food Crops (CRIFC), Indonesia

1995-1998

Project Goals

In the U.S. the European corn borer (ECB) (*Ostrinia nubialis*) is an important pest of maize, and maize transformation strategies using Bt genes have been highly effective in reducing losses to this insect. In Indonesia a close relative of the ECB, the Asian stem borer (ASB) (*Ostrinia furnacalis*). is the major pest. The life cycle of this insect allows it to threaten maize virtually all year round. The specific goals of this project were:

1. To produce tropical maize with resistance to Asian stem borer (ASB) (*Ostrinia furnacalis*).
2. Transfer of enabling technologies to Indonesian scientists via training in the US.
3. Commercialization of insect resistant germplasm generated from the project.

Project Impacts

The Garst/CRIFC collaboration produced was a technical success. Achievements included:

- Optimization of a cryV gene for expression in maize
- Demonstrated mortality of the Asian Corn Borer to the cryV protein
- Development of constructs for maize transformation utilizing the codon-modified cryV gene and the maize polyubiquitin promoter
- Transformation of tropical germplasm line PN2119 and temperate hybrid line A1888xB73 using the biolistic gun
- Transformation of the temperate hybrid line A1888xB73 using Garst's "whiskers" technology
- Field testing of transgenic A188xB73 lines for efficacy against the first and second generation European Corn Borer
- Of the 248 event-plan combinations, 39 exhibited first generation corn borer resistance as determined by a visual system of leaf damage assessment

Additionally, three Indonesian scientists were trained at Garst for five weeks to learn biosafety and regulatory issues. An additional four Indonesia scientists received technical training lasting from three to 12 months in which they learned:

- Maize transformation and regeneration
- Tissue culture of tropical and temperate germplasm
- Insect bioassays

- Molecular characterization; i.e. PCR, ELISA, Southern blots, Western blots
- Field evaluation, artificial infestation and statistical design/interpretation
- Industrial research and development

However, while a success from a research and capacity building point of view, the project was not ultimately successful in developing tropical maize for use in Indonesia. This was due to primarily policy constraints, but also additional technical constraints. While initially focused on using tropical germplasm, phytosanitary restrictions forced the project to initially transform a temperate line of maize. The legal uncertainty surrounding commercialization of maize developed using the biolistic gun required the use of ICI's proprietary technology, which was only successful in transforming one particular temperate line of maize. This material would have to be backcrossed into tropical maize for development of material suitable for Indonesia. Additionally, the Bt gene, which has been incorporated into the maize, was also proprietary.

At the time of the project, Indonesia did not have in place patent or plant variety protection laws that would protect hybrid seed and transgenic plants. Indonesia still cannot provide adequate legal protection for this material, although they have recently passed a Plant Variety Protection Law. Unfortunately none of these issues were brought to the table when the initial collaboration was undertaken. In this case, partners expressed reluctance to make commitments until the results of the research were known. Thus both the scientific and training component of the project proceeded with great success, but when the scientists returned home, no mechanism existed for them to transfer to their own country the genes and varieties with which they had worked at Garst.

At that time, Indonesia also lacked the appropriate biosafety guidelines or regulations for field-testing of genetically engineered plants, and many companies, as well as the ABSP, are reluctant to test material in countries without adequate biosafety policies. National guidelines were subsequently passed by ministerial decree on September 2, 1997, and with funding from the World Bank and the Indonesian government, construction of a biosafety containment facility began that year. There are currently several field trials of transgenic crops in the country, all of which have been produced by multinational companies.

The patent laws issues in Indonesia are still largely unresolved regarding protection of genes, and if this situation does not change it will continue to inhibit public sector research institutes from accessing proprietary materials from either the public or private sectors outside the country.

Although USAID and ABSP tried to pre-empt the policy issues that would affect the technology transfer process, additional levels of unforeseen detail were encountered that brought the process to a halt. In the case of Indonesia, the biosafety issues have now largely been overcome, but the questions of IPR still have to be resolved. The transgenic material produced during the project is held in trust, however the research contract with Garst has since expired and due to budgetary constraints was not renewed.

Indonesia Small Grants Program

The Indonesian Small Grants Program was a component of the ABSP collaboration with Indonesia, supported by USAID/Jakarta. The program, which funded grants up to an equivalent of $25,000, was open to both public and private sector institutions, and was designed to encourage the development of applied technologies of importance to Indonesian agriculture. Grants were solicited widely across Indonesian research institutes, universities and companies, and reviewed and approved by a Competitive Grants Review Committee. The Review Committee was composed of a co-ordinator from the State Minister for Scientific Research and Development based at CRIFC, and representatives from the industrial crops private sector, the horticultural crops private sector, the estate crops private sector, the university sector, the National Institute of Sciences R&D Center for Biotechnology, and the ABSP core program. The program was managed by CRIFC and the summaries below briefly outline those projects funded by the program. Total funding for the Small Grant Program was $100,000.

Use of immunoassay probe for detection and monitoring of *Phytophthora spp*., a causal agent of pod rot of *Theobroma* cacao

Biotechnology Research Unit for Estate Crops, Indonesia

Project Goal

Cocoa is an important crop in Indonesia with more than 58% of the 221,000-ton total production coming from smallholdings. The existing plant materials in Indonesia and other cocoa producing countries are susceptible to pod rot disease caused by *Phytophthora* spp. Forty percent of pod loss is usually from damage due to the disease and chemical control is currently the major control strategy. Fungal propagules in the soil are the primary cause of infection, but there are no techniques available to quantify the level of inoculum in the soil. Immunoassay has the potential to detect and quantify the pathogen population in the soil.
The objectives of this project were as follows:

1. To investigate if *Phytophthora* associated with cacao in Indonesia consists of several serotypes.
2. To produce polyclonal antibodies (PcAbs) against *Phytophthora* spp. associated with cocoa pod rot disease.
3. To investigate the potential of these antibodies for assessing inoculum potential of *Phytophthora* spp. in soil

Project Impacts

- The polyclonal antibody developed in this study was highly specific to the *Phytophthora* species associated with pod rot disease of cacao and did not cross react to other soil microorganisms.
- The antibody was further characterized and found to be bound to polysaccharides with molecular weights of 36, 25, 20 and 17 KDa.
- Dot Blot Immunosorbent Assay (DIBA) was determined to be an improved method for the detection and monitoring of *P. palmivora*, causal agent of pod rot disease of cacao.

Regeneration study of Indonesian sweet potato

Research Institute for Food Crops Biotechnology (CRIFC), Indonesia

Project Goal

Problems in sweet potato production in Indonesia are caused mainly by sweet potato weevil, and conventional breeding methods have so far failed to produce resistant material. The application of recombinant DNA technology therefore has potential to address this problem, however, this has been hampered due to the inability to regenerate plants efficiently.
The objective of this project was therefore:

- To study the *in vitro* culture ability and regeneration of seven Indonesian sweet potato cultivars.

Ginger micropropagation using bioreactor system

PT Fitotek Unggul, Indonesia

Project Goal

Ginger (*Zingiber officinale* Rose) is a herbaceous plant indigenous to Indonesia and widely spread throughout India and China. Ginger rhizomes are used as spices, in herbal medicine, and as raw material in the food, beverage and pharmaceutical industries. The demand for fresh and dry ginger and its essential oil on the world market is high both in domestic and international trade. Ginger is propagated through rhizomes and farmers usually take planting material from their own product, a practice that tends to spread diseases. Micropropagation methods have been developed for ginger and have made possible the supply of disease-free material year round. However, solid culture systems are limited in their ability to produce material in bulk, and therefore a bioreactor system of agitated liquid culture is recommended.

The main objective of this project was:

- To investigate the feasibility of producing ginger using a micropropagation bioreactor system.

The development of CVPD free citrus seedling from protoplast fusion and embryogenic callus cultures

Gadjah Mada University, Indonesia

Project Goal

Citrus greening disease is a major cause of crop and tree loss in many parts of Asia and Africa. Before it was identified as one disease, it was known by various names: yellow shoot (huanglungbin) in China; likubin (decline) in Taiwan; dieback in India; leaf mottle in the Philippines; citrus vein phloem degeneration ((CVPD) in Indonesia; and yellow branch, blotchy-mottle, or greening in South Africa. As it became clear that all these were similar diseases the name "greening" has been widely adopted. Losses due to greening are not easy to assess but are high in many citrus growing areas. Sometimes only sectors of a tree are affected and losses are small, but in other cases the entire tree is infected and crop loss is total. No detailed loss studies have been published, but in Indonesia not less than 3 million trees were destroyed between 1960 and 1970, with groves in most regions of Java and Sumatra being abandoned by 1983.

Greening is caused by an unculturable Gram-negative phloem limited bacteria belonging to the alpha subdivision of the *Proteobacteriaceae*. The 16S rDNA comparative studies led to the proposed classification of the causal agent as a "*Candidatus*" with generic name *Liberobacter*, as defined for

uncultured organisms. Two distinct species have been identified based on sequence comparison and the names, *Liberobacter africanum* and *Liberobacter asiaticum*, have been proposed for the African and Asian greening organism respectively.

The objective of this project was to:

- Use tissue culture techniques to obtain citrus seedlings free of the CVPD agent.

Project Impacts

- The plant material that gave the best explant material for embryogenic nucellus cell culture was determined to be immature fruits.
- Optimal culture media for callus culture and regeneration were identified for several citrus varieties.

Further funding for this project was obtained from The Indonesian Directorate General of High Education.

Indonesia Biosafety

Activities

➤➤ Public Awareness/Acceptance of Biotechnology in Indonesia

Over the past five to six years, ABSP has assisted a number of CRIFC faculty and staff in both biotechnology technical training and in regulatory and intellectual property policy training. This training has provided the researchers at CRIFC with sound scientific knowledge to engage in current biotechnology education and awareness campaigns throughout Indonesia. Teams of scientists recently traveled in Central Java, West Java and Bali to present seminars on the genetic engineering for crop improvement and Indonesia's regulatory system for GMOs. The audience was primarily university and research institute personnel. CRIFC plans to expand this effort to include East Java and will conduct training to educate additional scientists on communicating biotechnology issues and policy to the Indonesian public.

➤➤ Assistance to Indonesia in Developing National Biosafety Guidelines

Indonesia has been a major focus country for ABSP's capacity building in biosafety and intellectual property rights. In 1995, ABSP began providing Indonesia with assistance in developing its national biosafety guidelines.

- ➤➤ A consultant from the USDA National Biological Impact Assessment Program worked as a special consultant for ABSP and assisted the committee formed by CRIFC in drafting the guidelines. Indonesian experts in each of three research sectors (plants, animals, and microorganisms) were selected as the writing committee with the approval of Indonesia's Ministry of Agriculture. A first draft was produced and entitled *"Guidelines for Planned Introductions into the Environment of Organisms Genetically Modified by Recombinant DNA Techniques*."
- ➤➤ In order to improve upon this first draft, CRIFC and ABSP organized a biosafety workshop, held in May 1996, and a total of 45 participants from both the public and private sector attended. Based on the workshop, a new draft was produced, and the guidelines for biosafety were proposed as the basis of a decree from the Minister of Agriculture.
- ➤➤ A second workshop was then held to finalize the second draft, which was reviewed by Indonesian officials and the Bureau of Law at the Indonesian Ministry of Agriculture.
- ➤➤ National guidelines were subsequently passed by ministerial decree on September 2, 1997
- ➤➤ Field tests were approved in 1998 by the National Biosafety Committee for Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean. Multi-locational, unconfined trials have been conducted.

Impacts

Indonesia has been a major focus country for ABSP's capacity building in biosafety. Through workshops, internships, and consultants, Indonesian scientists and policy makers have been brought together to address regulatory issues relating to the testing and commercialization of transgenic crops. Along with Egypt, Indonesia has made significant progress in the development of biosafety guidelines and procedures.

➤➤ Biosafety Guidelines Developed and Approved

National biosafety guidelines were passed by ministerial decree on September 2, 1997, allowing Indonesian scientists and international companies and research institutions to field test transgenic crops in Indonesia.

➤➤ **Transgenic Crops Commercialized.**
Indonesia's Biosafety Committee has now given deregulated status to 5 transgenic crops (Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean from Monsanto) for unconfined multi-location trials. Later in 1999 they plan to conduct confined field trials of Bt corn from Pioneer Hi-Bred, and other crops are currently being tested in the greenhouse. The Ministry of Agriculture recently gave its approval to the limited sale of transgenic cotton. An application for a confined field trial of ABSP's potato tuber moth resistant Bt potato was submitted to the Indonesian Biosafety Committee and USAID's Biosafety Committee in 2000, and a field trial is planned as soon as approvals are granted.

➤➤ **GMO Food Safety Guidelines Developed.**
Supported by ABSP, Dr. Muhammed Herman and Dr. Achmad Hidayat, Central Research Institute for Food Crops (CRIFC) attended the International Food Safety course at Michigan State University (MSU) in 1999. Drs. Herman and Hidayat were subsequently appointed to the committee charged with drafting Food Safety Guidelines for GMOs in Indonesia. The Ministry of Agriculture and other relevant ministries in Indonesia have since approved these guidelines.

Indonesia IPR/Technology Transfer

Activities

➤➤ **Intellectual Property/Patent Internship Program, Stanford University, April 1993.**
An IPR internship program was designed and implemented by Professor John Barton of Stanford Law School from April 1-30, 1993. Seven interns from Egypt, Kenya and Indonesia participated in the program, the goal of which was to provide hands-on experience to legal and scientific personnel from developing countries in various issues related to intellectual property rights. In addition the internship enhanced communication between those involved in the sciences and those with responsibilities in the legal issues surrounding biotechnology. The program encouraged the assessment of current intellectual property structures within the participants' home countries, provided access to literature and expertise regarding IPR in both the public and private sectors.

➤➤ **IPR Workshop, Washington DC, July 1994.**
ABSP sponsored this workshop in Washington DC from July 11-14, 1994 as a follow up to the Egypt workshop. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka as well as a number of institutions and agencies such as USAID and the World Bank. The purpose of the workshop was to present intellectual property rights in biotechnology as an important issue to institutions and individuals. Proceedings of this workshop were published: *Intellectual Property Rights, Proceedings from the ABSP Workshop Series July 11 - 14, 1994, Washington, D.C.*

➤➤ **Plant Variety Protection and Patents Workshop, Indonesia, 1996.**
ABSP, through the support of USAID/Jakarta organized a two-day workshop on intellectual property rights in agriculture from March 25-26, 1996, which was attended by fifty senior

representatives from the government and private sector in Indonesia. The workshop was organized in collaboration with the Central Institute for Food Crops (CRIFC) in the Indonesian Ministry of Agriculture. The main goal of the workshop was to assist Indonesia in drafting their new plant variety protection law.

Impacts

➤➤ Establishment of Technology Transfer Office

Through training and technical assistance from ABSP, the Agency for Agricultural Research and Development (AARD; equivalent to the Agricultural Research Service/USDA) established a new office of Intellectual Property and Technology Transfer in Bogor, Indonesia, July 1999. The ABSP office trained two staff members in various issues of IP management and technology transfer. The office (known by the Indonesian acronym KIAT) is now actively involved in educating scientists and policy makers in Indonesia in management of IP. KIAT is also working with the private sector to license technologies generated within AARD institution and will serve as the main focal point for management of intellectual properties related to agriculture/biotechnology. The AARD is one of the few developing country institutions to recognize the benefits of intellectual property and to develop within the ministry a system for protecting and exploiting Indonesian innovations to benefit Indonesian agriculture. Within 3 months of its operation, KIAT has executed 5 license agreements to commercialize a wide range of technologies developed by the AARD institutions.

According to Dr. Acmad Fagi, Secretary General of the AARD, this office is the direct result of training received in IPR and Technology Transfer at MSU via the short course. The office will have a legal and financial division, general business division, technical division, and a secretariat. Ketty Karyati, who has received training as part of ABSP's capacity building efforts with Indonesia, will be the administrator of the office as the secretary. KIAT has expressed interest in running an in-country IPR workshop to educate key scientists and various AARD institutions. In addition, MSU's draft IP policy was shared with KIAT to be used as a basis for developing a system-wide policy in IP.

➤➤ Founding of the Indonesian Inventor Society.

Dr. Didiek Hadjar from the Estate Crops Research Institute attended the MSU IPR and Technology Transfer Course in 1998. He has since co-founded a new organization called the Indonesian Inventor Society and is serving as President. Again, this organization was developed as a direct result of Dr. Didiek's participation in the course. There have been several biofertilizer/biofungicide technologies patented with the assistance of this organization and are in various stages of commercialization.

➤➤ Indonesian PVP Law passed in 2001.

In December 2000, the Indonesian Parliament approved the Plant Variety Protection (PVP) Act. This law is based on the UPOV 1991 Convention. ABSP assisted in drafting this new PVP law in 1995, and researchers trained by ABSP have been working with the Minister of Agriculture to educate the Parliament about the law.